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Advances in our understanding of the pathogenesis of Haemolytic Uremic Syndromes.

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Abstract

Hemolytic uremic syndrome (HUS) is major global health care issue as it is the leading cause of acute kidney injury in children. It is a triad of acute kidney injury, microangiopathic hemolytic anemia, and thrombocytopenia. In recent years, major advances in our understanding of complement-driven inherited rare forms of HUS have been achieved. However, in children 90% of cases of HUS are associated with a Shiga toxin-producing enteric pathogen. The precise pathological mechanisms in this setting are yet to be elucidated. The purpose of this review is to discuss advances in our understanding of the pathophysiology underlying HUS and identify the key questions yet to be answered by the scientific community.

Keywords Haemolytic Uremic Syndrome, Thrombotic microangiopathy, Complement.

Introduction

Haemolytic uremic syndrome (HUS) is a major global health care issue as it is the leading cause of acute kidney injury in children. It is a triad of acute kidney injury, microangiopathic haemolytic anaemia and thrombocytopenia; first described in 1955 by Gasser et al. (1). Over ninety percent of childhood HUS cases are associated with infection (2). Shiga toxin-producing *Escherichia Coli* found in contaminated food and water supplies is typically the infective trigger; although HUS has also been reported following exposure to *Shigella*, *Campylobacter* and *Streptococcus Pneumoniae* (2, 3). In the United States Shiga toxin associated HUS affects 0.5-2.1 people per 100,000 population per year. The majority of these cases are reported in children under 5 years of age in whom the incidence is considerably higher at 6.1 per 100,000 population per year (4).

The remaining ten percent of HUS cases are termed ‘atypical’. These include familial cases which are due to genetic abnormalities in complement regulation and other non-infective causes such as pregnancy, drugs, malignancy, connective tissue disorders and transplantation (5). Over the last decade there has been a renewed appreciation and considerable interest in the role of complement system in renal disease which has been compounded by the effectiveness of Eculizumab (a monoclonal humanised antibody against C5) in the treatment of atypical HUS. Since 2009, when Eculizumab was first used in atypical HUS, there have been numerous case reports detailing the success of the drug in controlling the disease. A landmark prospective Phase 2 trial published in 2013 further consolidated the evidence for significant improvement in renal function in atypical HUS patients treated with Eculizumab (6, 7).

The diagnostic challenge for the Nephrologist therefore, is to differentiate between atypical HUS and infection associated HUS to facilitate rapid, targeted treatment with Eculizumab if indicated. This review will discuss the classification, diagnosis and treatment of HUS; current understanding of the pathophysiology underlying the condition and the key questions yet to be answered by the scientific community.

Classification of HUS

HUS is categorised histopathologically as a thrombotic microangiopathy (TMA). This term was first introduced in 1952 by Symmers to describe: capillary wall thickening, swelling and detachment of the endothelial cell from the basement membrane, accumulation of debris in the subendothelial space and intraluminal platelet thrombosis; culminating in partial or complete obstruction of the vessel (1). When this process occurs in the microvasculature of the kidney (as is the case in HUS) renal impairment is seen due to organ ischaemia. In contrast, in thrombotic thrombocytopenic purpura (TTP) neurological impairment is observed as the predominant microvascular bed affected is the brain (2). The microangiopathic haemolytic anaemia that ensues is a consequence of erythrocyte sheer stress resulting in red cell fragmentation. Platelet activation and entrapment in microthrombi as well as trafficking to the reticuloendothelial system leads to thrombocytopenia, local thrombosis and organ ischaemia (2, 8). Thus, TMA represents a final common pathway in a number of disease processes which amongst others include HUS and thrombotic thrombocytopenic purpura (TTP). See Figure 1. The clinical sequelae observed is dependent upon the vascular bed and organ affected (2). Both conditions are a consequence of endothelial cell injury but are now accepted to be different diseases with distinct underlying mechanisms (9).

TTP is a pentad of microangiopathic haemolytic anaemia, thrombocytopenia, fever, acute kidney injury and neurological features (9). It is due to a deficiency in the von Willebrand factor cleaving enzyme ADAMTS13 (A Disintegrin and Metalloprotease with ThromboSpondin motif repeats 13); or inhibitory autoantibodies (10, 11, 12). Historically, a decrease in ADAMTS13 activity has been considered to be specific for a diagnosis of TTP (13). However, it is becoming increasingly apparent in recent case reports that reduced levels of ADAMTS13 are seen in patients with other forms of TMA including atypical HUS. Furthermore, in TTP patients there is evidence of C3 consumption and increased alternative complement pathway activation. Work by Noone et al. using ex-vivo models has suggested a potential role for von Willebrand factor multimers as complement regulators on the surface of endothelial cells (11, 13). Given the overlap in clinical presentation and the emerging similarities in possible underlying mechanisms distinguishing between HUS and TTP is becoming increasingly challenging for the physician (14). Given the latest advances in therapeutic options for atypical HUS and the need for future studies into the treatment of TMA it is vital that an accurate diagnosis is made both for the individual patient; but also at a population basis to allow appropriate stratification in clinical trials (15).

The Complement Cascade

The complement system has been implicated in a number of renal diseases and represents an area of great interest within the Nephrology community at present (16). It is well established that atypical HUS is caused by impaired regulation of complement activation on glomerular endothelial cell surfaces (17, 18). Complement hyperactivation following Shiga toxin exposure has also been proposed as a mechanism in the development of infection associated HUS although this yet to be proven (19). A recent study from Ozaki et al. has demonstrated that inhibition of the mannose binding lectin (MBL) complelment pathway protected mice against renal injury due to Shiga toxin exposure (20). Hence, it is vital to

have an understanding of the complement pathway before delving into the pathophysiology of HUS.

The complement pathway forms part of the innate immune response and consists of 60 proteins which play a vital role in the host defence against pathogens and in the maintenance of tissue homeostasis (19, 21). These proteins can be plasmatic (fluid) or membrane bound receptors (solid) (2, 22). Three pathways leading to activation of the complement cascade have been described: classical, lectin and alternative. All culminate with the formation of the membrane attack complex (MAC) leading to the insertion of pores in the target cell membrane resulting in osmotic lysis and cell death (19, 23) Figure 2. The classical pathway is activated by binding of C1q to antibody-antigen complexes. The lectin pathway is triggered by binding of MBL to mannose containing carbohydrates on microbial surfaces which activates MASP 1 (mannan-binding lectin serine protease 1) and MASP 2 (19, 23). Both the classical and lectin pathways lead to the generation of the same C3 convertase C4b2a on target cell surfaces resulting in proteolytic cleavage of C3 into C3a and C3b (19, 22).

The alternative pathway differs from the other two in that it is continually active at low levels in the plasma. Spontaneous hydrolysis of the thioester bond in C3 induces a conformational change in the protein which facilitates binding of complement Factor B. Once bound to C3, Factor B is cleaved by Factor D to Ba and Bb. This generates the alternative pathway C3 convertase C3Bb; resulting in proteolytic cleavage of C3 into C3a and C3b (see Figure 2) (19, 22, 23). Association of C3b with C3 convertases (generated by any of the 3 pathways) leads to formation of C5 convertases which cleave Factor C5 into C5a and C5b; initiating the assembly of the MAC on target cell surfaces (19). This terminal pathway in the complement cascade also acts to alert the host defences by the release of chemoattractant and inflammatory mediators; C3a and C5a are potent anaphylatoxins that recruit phagocytes and induce endothelial cell activation (19, 24).

The alternative pathway acts as a positive feedback loop – amplifying complement activity. C3b on target cell surfaces generated by the classical or lectin pathways acts as a site of C3Bb formation and the activation of alternative pathway itself leads to deposits of C3b on cell surfaces – further potentiating this loop (23). As such the alternative pathway may account for up to 80% terminal pathway activity according to Blatt et al (23). Hence, the complement cascade has great potential for non-specific destruction, which host cells must be protected from. Indeed, complement activation is regulated by a number of plasma and membrane bound factors (19, 22).

Complement Regulation

It is essential that complement activation is regulated to prevent excessive cell injury and inflammation (17, 23). Host cells are protected by surface bound and soluble plasma complement regulatory proteins. Of particular importance are the plasma proteins Factor H and Factor I which negatively regulate the C3b amplification loop. Although found in the plasma both can act in the fluid phase and on cellular surfaces. Their role on cell surfaces that lack membrane bound complement regulators e.g. the glomerular basement membrane in the kidney is vital as they are the only defence against complement attack and thrombus formation (17, 24).

Notable membrane bound complement factors include CD46 (MCP – membrane co-factor protein), CD55 (DAF – decay activating factor), CD59 and CR1. By definition these surface bound complement regulatory proteins are restricted in activity by their cellular expression

and distribution; the exception being CR1 which is expressed by neutrophils, lymphocytes and erythrocytes which circulate throughout the body rather than being tissue bound (24, 25). Atypical HUS is known to be primarily due to hyperactivation of the alternative complement pathway as a result of loss of function mutations in complement regulatory factors or gain of function mutations in C3 or Factor B (2, 5). Autoantibodies against regulatory complement factors have also been described in atypical HUS and with high prevalence in haematopoietic stem cell transplantation associated TMA (17). It was the discovery by Warwicker et al. in 1998 that a mutation in Factor H led to the development of atypical HUS, which prompted the search and subsequent discovery of over 120 mutations in complement regulatory genes responsible for atypical HUS (19). It is this link that has prompted the investigation of the role of complement in other forms of TMA.

Atypical HUS

Accounting for ten percent of HUS cases atypical HUS is a rare condition. However, it is associated with significant morbidity and mortality; leading to end-stage renal disease in around half of patients affected (17). Inheritance may be sporadic, autosomal recessive or autosomal dominant with incomplete penetrance (25). Interestingly, identification of the specific gene polymorphisms resulting in abnormalities of the complement cascade (accounting for up to 70% of atypical HUS cases) provide powerful prognostic information allowing patients to be counselled about likelihood of post-transplantation recurrence (5, 19, 26). Mutations in Complement Factor H are associated with the worst outcomes, whereas those with MCP mutations do far better (19). A minority of atypical HUS cases have been attributed to the dysfunction in degradation of von Willebrand factor, thrombomodulin mutations or loss of function mutations in diacylglycerol kinase ε (DGKε) (26).

The landmark discovery in 2013 by Lemaire et al. that recessive mutations in DGKε cause atypical HUS in a significant proportion of children in the first year of life, has led to an alternative pathophysiological mechanism being proposed for the condition (27). Prior to this all previous mutations were associated with unrestricted alternative pathway complement activation. Diacylglycerol kinases (DGKs) are intracellular lipid kinases that phosphorylate diacylglycerol (DAG) to phosphatidic acid (PA) leading to termination of DAG signalling (28). The DGKε isoform preferentially phosphorylates arachidonic acid containing DAG (AADAG) to PA thereby terminating AADAG signalling (28). AADAG is a key intracellular signalling molecule that activates protein kinase C (PKC). AADAG-dependent PKC signalling promotes thrombin-induced platelet activation. In endothelial cells it has been shown to increase production of prothrombotic factors such as von Willebrand factor and tissue factor; as well as downregulating VEGFR2 signalling, which has been linked to TMAs previously (27, 29). Bruneau et al. have demonstrated in endothelial cells that knockdown of DGKε results in a proinflammatory and prothrombotic state via over activation of p38 and p44/42 MAP kinases (28). Hence, the hypothesis that loss of function mutations in DGKε results in sustained AADAG signalling and a subsequent prothrombotic state. See Figure 3.

In podocytes DAGs have been shown (REF) to modify slit diaphragm function and through PKC-dependent pathways downregulate expression of the VEGF receptor VEGFR2 (or Flk-1). Podocytes are the major source of VEGF which is essential for maintaining the nearby glomerular endothelium. Quaggin et al. have shown that disruption of VEGF signalling in podocyte-specific VEGFA knock-out mice results in a glomerular TMA with a phenotype that recapitulates that seen in HUS. In support of this finding cancer patients treated with the VEGF inhibitor bevacizumab have also been reported to develop glomerular TMA (30). Although DGKε deficiency was initially thought to cause atypical HUS through mechanisms

distinct from complement dysregulation; there is mounting evidence that complement activation products may alter DGKε regulated pathways in podocytes leading to proteinuria. Interestingly, DAG directly activates TRCP6 in the podocyte via a PKC-independent mechanism. Mutations leading to hyperactivation of TRPC6 channels are known to result in podocyte effacement and inherited forms of nephrotic syndrome (8). Remuzzi et al. have reported that proteinuria is more common than initially perceived in atypical HUS. This appeared to be more prevalent in children with the condition - although larger numbers of cases would need to be reviewed to definitively address this trend. Certainly, glomerular epithelial cells could be key in the development of atypical HUS and the interaction of complement with the DAG-PKC pathway warrants further investigation (8).

Complement Blockade

Prior to the advent of the use of Eculizumab a humanised, mouse anti-C5 monoclonal antibody; the only treatment option for atypical HUS was plasma exchange and supportive therapy in the form of dialysis (31). In contrast to TTP where the response to plasma exchange is in the region of 80-90%; atypical HUS patients undergoing plasma exchange may see initial improvement in platelet count and anaemia but only transiently; with the underlying TMA and complement activation persisting (31, 32). Indeed, end-stage renal disease or death occurs in up to 40% of atypical HUS patients at first presentation despite plasma exchange (7). Eculizumab has transformed treatment for these patients and is increasingly being reported to be efficacious in cases of HUS associated with medications, pregnancy and connective tissue disorders such as systemic lupus erythematosus (SLE) (33-36).

Eculizumab binds with high affinity to C5 and blocks the generation of anaphylatoxin C5a and the assembly of the MAC (C5b-C9) (7). As such it is a terminal complement pathway inhibitor and leaves proximal functions of immune clearance intact (31). The blockade of C5 however, does increase susceptibility to meningococcal infections. This has prompted the recommendation when prescribing Eculizumab, to ensure that patients are vaccinated as soon as possible before initiation of treatment. Patients are informed and consented about this risk and monitored for the early signs of the infection which can prove fatal (37).

Following two prospective Phase 2 Trials reported in the NEJM in 2013 by Legendre et al. the FDA approved Eculizumab for the treatment of atypical HUS. These trials included patients with atypical HUS aged 12 years and above with thrombocytopenia and renal dysfunction (Trial 1); or renal dysfunction alone without a significant reduction in platelet count during plasma exchange (Trial 2). Each group received Eculizumab for 26 weeks and a subsequent extended period. Both trials demonstrated that Eculizumab treatment inhibited complement-mediated TMA and this effect translated clinically to substantial recovery in renal function and haematological parameters; with earlier intervention associated with greater clinical benefit (7).

The success of Eculizumab in the treatment of atypical HUS is reflected in expert consensus (including NICE approval in the UK – currently the most expensive treatment endorsed by this National guidance body); which advocate early use in patients with atypical HUS so as to achieve optimal chance of renal recovery (38). See Table 1. The question remains as to the necessary duration of Eculizumab treatment for patients with confirmed atypical HUS and as to whether its use should be extended to other forms of TMA (39).

Infection Associated HUS

By far the commonest cause of HUS, infection associated HUS accounts for ninety percent of cases (2). Within this group the majority follow exposure to contaminated food and water with Shiga toxin producing *Escherichia Coli* (*E.coli*) (3). Conventionally this form of HUS has been known as 'typical' or 'diarrhoea associated'. However, these terms have proved unhelpful given that up to half of patients with atypical HUS can present with an initial diarrhoeal illness. This only adds to the complexity of distinguishing between atypical HUS and Shiga toxin associated HUS on clinical grounds alone (38). The diagnosis of atypical HUS through identification of a mutation in complement regulation by DNA testing is time consuming and delays diagnosis. Moreover in up to 50% of cases of atypical HUS no predisposing polymorphism is currently detectable (19, 40).

Given the need for clarity in terminology, the definition STEC HUS is now used to describe HUS secondary to Shiga toxin producing *Escherichia Coli*. This is distinct from other infective causes of HUS such as *Streptococcal pneumoniae*-related HUS which is considerably rarer, more severe and due to invasive pneumococcal disease (41). This bacteria produces a neuraminidase that exposes the Thomsen-Friedenreich antigen (T-antigen) normally present on red blood cells, platelets and glomerular endothelial cells in the host; thus rendering these cells susceptible to antibody attack resulting in microangiopathic haemolytic anaemia, thrombocytopenia and acute kidney injury (41, 42).

Diagnosis of STEC HUS is reliant upon faecal testing in the laboratory using stool culture, immunoassays and PCR (polymerase chain reaction) assay to detect Shiga toxin genes. These tests together will identify up to 70% of cases (43). The diagnosis of atypical HUS is therefore often implied by the absence of STEC. As a result patients presenting with a microangiopathic anaemia, thrombocytopenia and acute kidney injury are commenced on plasma exchange. Once TTP has been excluded (ADAMTS 13 activity above 5-10%) and if STEC testing is negative then a diagnosis of atypical HUS must be considered likely and Eculizumab started (31).

The pathophysiology underlying STEC HUS is yet to be determined and as such there are currently no specific treatments for the disease other than supportive care (20). Acute mortality outcomes for STEC HUS have improved with the introduction of early dialysis from 30% to 5%. However, of those patients that survive the initial insult up to 30% develop proteinuria; and 18% will go on to develop chronic renal failure (44). A better understanding of the pathophysiology underlying STEC HUS is essential if targeted therapy for this condition is to be developed.

Shiga toxins are exotoxins produced by *E. coli*, as well as by other bacteria including *Shigella dysenteriae* and *Campylobacter jejuni* (2, 45). They consist of a single 30 kDa enzymatic A subunit in non-covalent association with five identical B subunits of 7 kDa each (3, 46). The genes encoding Shiga toxin are found within lambdoid bacteriophages present in all pathogenic STEC. Toxin secretion occurs by phage-mediated bacterial lysis. (3, 45). It is the B subunit of Shiga toxin that binds to the glycosphingolipid receptor Gb3. It has been shown that cells lacking Gb3 expression are resistant to the toxic effects of Shiga toxin; meaning the pattern of expression of Gb3 in different cell types is a reliable predictor of Shiga toxin site of action (3, 47).

Unlike human glomerular cells, murine glomerular endothelial cells and podocytes do not express Gb3 (47). As a result, development of rodent models of STEC HUS that recapitulate the glomerular TMA lesions seen in humans with the disease have not been possible. Mice challenged with Shiga toxin alone (orally or intraperitoneally) develop lethal tubular disease

without histological evidence of glomerular TMA. A mouse model reported by Keepers et al. involving administration of both Shiga toxin and lipopolysaccharide was shown to cause some evidence of thrombus formation in glomeruli but the predominant lesions were tubular and led to dehydration; a phenomenon that is not seen in human disease (48). Clearly, Gb3 is the cellular target of Shiga toxin and in further support of this Okuda et al. have demonstrated constitutive whole body Gb3 knockout mice are completely protected from the lethal effect of Shiga toxin exposure (49).

Following binding to Gb3, Shiga toxin is endocytosed and transported in a retrograde manner from the endosome to the trans-Golgi network. From here it is transported to the endoplasmic reticulum (ER), where using the ER-associated degradation machinery it is able to translocate into host cell cytoplasm (45, 50). Shiga toxin is known to act in three main ways: firstly it inactivates ribosomes by enzymatically removing an adenine residue from 28S ribosomal RNA. This triggers the ribotoxic stress response leading to MAPK signalling and activation of cytokines and chemokines that result in proinflammatory and proapoptotic pathways (3, 46). Secondly, the unfolded protein response (UPR) may be triggered by Shiga toxin unfolding within the ER. Prolonged signalling via the UPR will induce apoptosis in cells (46). Finally, the binding of the B subunit itself can initiate a cytoplasmic transduction cascade that is distinct from the ribotoxic stress response but that also culminate in a prothrombotic, proinflammatory cellular environment (3, 46, 51). Attempts made to block Shiga toxin binding with synthetic Shiga toxin binders such as STARFISH or Synsorb-Pk; or to inhibit intracellular transport of the toxin with molecules such as Exo1 and Golgicide A have to date been ineffective (45).

Evidence of complement activation in STEC HUS

Over the last decade, there has been increasing evidence in support of a role for complement activation in Shiga toxin associated HUS. Some patients with STEC HUS have been reported to have low levels of circulating C3 and evidence of increased levels of C3 convertases and Factor B (19). Furthermore, a recent publication by Ozaki et al. has reported a protective effect of a monoclonal antibody against MBL in STEC HUS (20). This has led to new insights into the role of complement activation in Shiga toxin induced renal injury. However, our understanding of the precise pathophysiological mechanisms underlying the disease is far from complete. The literature is divided as to whether C5 inhibition with Eculizumab is beneficial in STEC HUS (20). This has been complicated by the lack of any randomised controlled trials. Attempts at retrospective analysis of patient outcomes in outbreaks of STEC HUS such as that seen in Germany in 2011 have been made. However, unravelling any meaningful answers as to the effectiveness of Eculizumab in such studies is very difficult as only the most severely affected patients that administered the treatment and it is unclear as to whether they would have recovered without it (52). Further work both in the laboratory and in the clinic is needed to aid our understanding of the mechanisms responsible for STEC HUS.

Conclusions

Complement pathway hyperactivation is emerging as a potential common mechanism underlying the pathogenesis of glomerular TMA. Identification of the genetic mutations in complement regulatory proteins, C3 convertase components and anti-CFH antibodies in atypical HUS has led to the advent of a new era of pharmacological complement modulation (19). Indeed, the success of Eculizumab in the management of atypical HUS has

transformed the management of these patients and resulted in reduced mortality and a reduction in progression to end stage renal failure (6).

Furthermore, support for role of complement in the pathogenesis of secondary HUS is provided by recent reports of the efficacy of Eculizumab in the treatment of HUS associated with drugs, pregnancy and SLE (33-36). However, the heterogeneity of these groups and the discovery that some individuals have an underlying genetic pre-disposition (due to mutations in complement regulators) merely adds to the difficulty in determining the true benefit of complement blockade in such cases (19).

The use of Eculizumab in STEC HUS remains controversial and further research is needed to elucidate the pathophysiological mechanisms underlying this form of HUS. Delineating the role of complement, which pathways are involved and the cellular target of Shiga toxin in the glomerulus will be key. The development of a murine model that more accurately recapitulates Shiga toxin HUS in the human would mean improved evaluation of any potential benefit of complement blockade in the disease prior to clinical trials in humans (53).

The challenge for the future lies in identifying the particular pathophysiological mechanisms responsible for the non-genetic forms of HUS and subsequent development of specific treatment strategies for this devastating disease.

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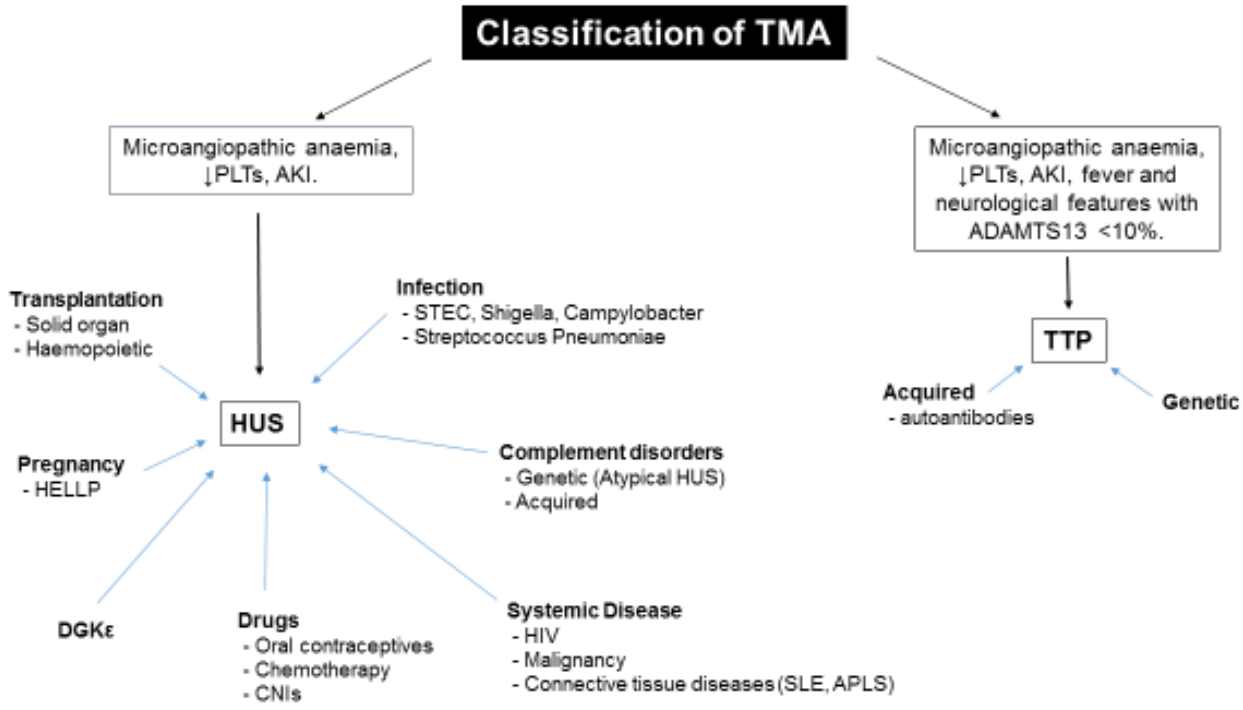
Figure 1: Classification of Thrombotic Microangiopathy (TMA).

It is now widely accepted that classification of TMA according to aetiology (rather than clinical features) is a more useful guide to prognosis and treatment. TMA represents a final common pathway in many disease processes (2).



PLTs – platelets; HUS – Haemolytic uremic syndrome; AKI – acute kidney injury; CNI – calcineurin inhibitors; SLE – systemic lupus erythematosus; APLS – anti-phospholipid syndrome.

Figure 2: The Complement Cascade.

Three pathways have been described leading to activation of the complement pathway; classical, lectin and alternative. All culminate with the formation of the membrane attack complex (MAC) leading to the insertion of pores into the target cell membrane resulting in osmotic lysis and cell death. The alternative pathway differs from the other two in that it is continually active at low levels in the plasma and is activated by spontaneous hydrolysis of a thioester bond in C3. To protect host cells from non-specific destruction complement activation is regulated by a number of plasma (CFH, CFI) and membrane bound factors (19, 22, 23).

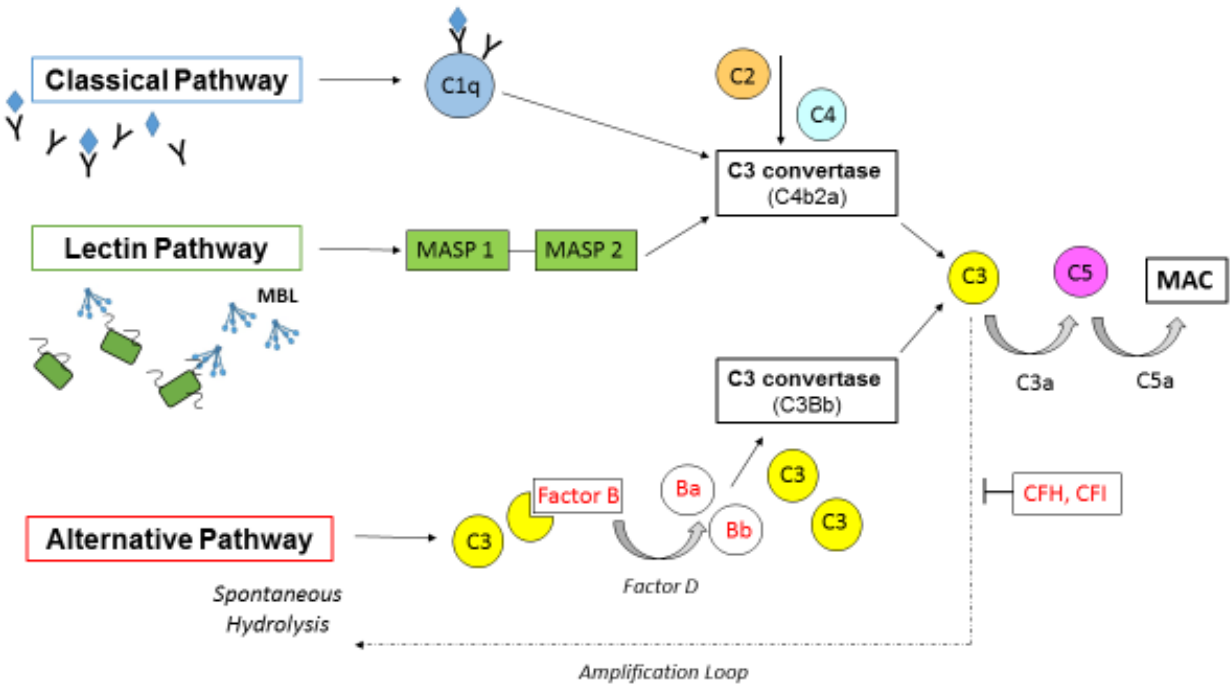


Figure 3: The effect of AADAG signalling.

DGKε is an intracellular kinase that preferentially phosphorylates AADAG to PA thereby terminating AADAG signalling. Loss of function mutations in DGKε lead to unregulated AADAG signalling and PKC dependent platelet activation and increased production of pro-thrombotic factors (27, 28, 29).

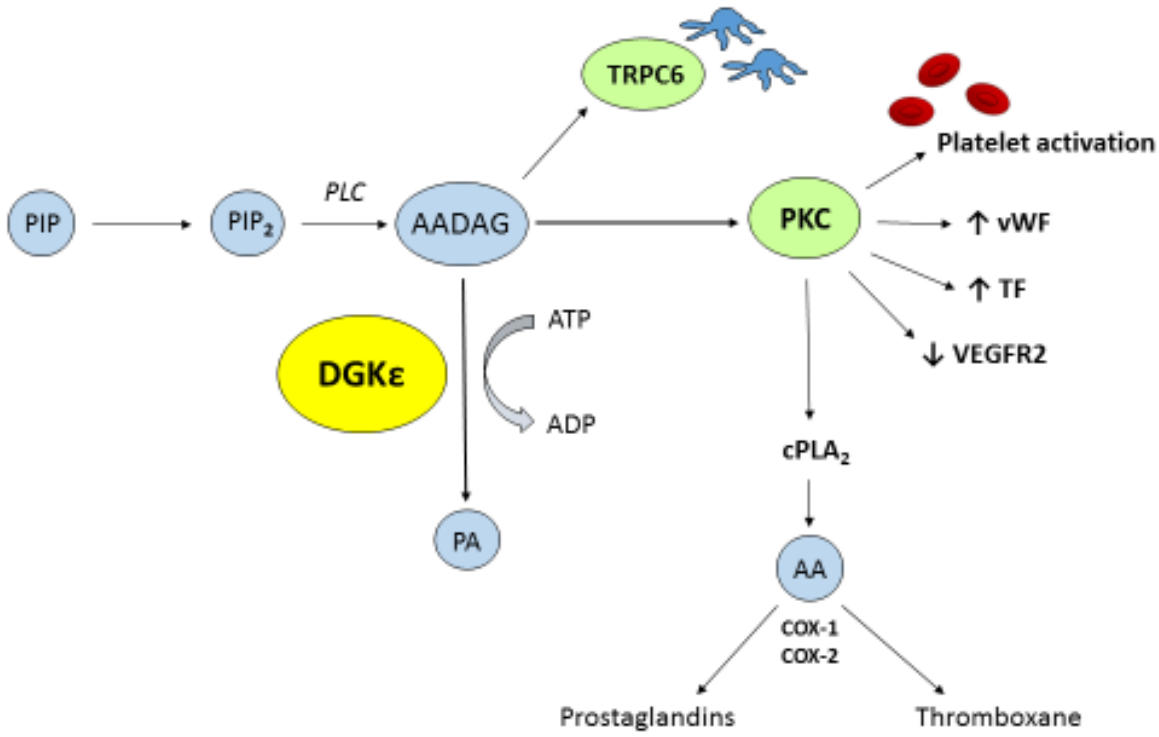


Table 1: Diagnosis and Management of Thrombotic Microangiopathies (TMAs).

Type of TMA	Diagnosis	Management
TTP	Pentad of clinical symptoms (thrombocytopenia, haemolytic anaemia, AKI, fever and neurological features). ADAMTS13 <10%	Plasma Exchange (PEX) Steroids Consider Rituximab
Infective HUS (STEC, *Shigella*, *Campylobacter* HUS; *Pneumococcal* HUS)	Triad of thrombocytopenia, haemolytic anaemia and AKI). Stool culture Positive Serology ADAMTS13 >10%	Supportive – may require renal replacement therapy.
Atypical HUS	Triad of thrombocytopenia, haemolytic anaemia and AKI). Measurement of complement regulatory factors (CFH, CFI and MCP) and C3/C4. Genetic Screening for complement mutations. ADAMTS13 >10% Stool culture and serology negative and infective cause not suspected.	PEX Eculizumab Consider steroids if anti-Factor H autoantibodies present.
Transplantation	Prophylaxis in known atypical HUS mutations De novo atypical HUS	PEX Eculizumab Rituximab to be considered if autoantibodies to Factor H present. In CFH mutations consider combined liver – kidney transplantation.